

## EXPERIMENTS CONCERNING THE MECHANISM OF PITUITARY COLLOID SECRETION

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ONE PLATE (FIVE FIGURES)

Although a definite intermediate or middle lobe is not distinguishable in the adult human pituitary the borderline region between the anterior and posterior lobe is occupied by numerous cysts whose irregular cavities are filled by colloid. This homogeneous material is similar to that seen in the pituitary cleft between the anterior and middle lobe of the adult rodent hypophysis. The cysts are mainly derived from the cleft in Rathke's pouch, a cleft which is usually present in infants and occupies most of the space inside the middle lobe. In most cases it is practically obliterated at puberty and in the adult the above mentioned small cysts take its place (Fraser, '21; Schönig, '26; Marburg, '29). While the cleft persists there is usually a distinct difference in the epithelium of its anterior and posterior wall. The anterior wall consists of three to four layers of cells, all of which show the characteristics of anterior lobe cells, some of them containing eosinophilic, others basophilic granules. The posterior wall, however, is usually lined with a simple layer of cuboidal or cylindrical ciliated cells. In cases in which a number of small cysts is present instead of a large one, the epithelium may be composed either of anterior lobe cells or of short cylindrical cells with or without cilia (Rasmussen, '29). If the cysts are very much distended with colloid, the lining becomes flat (Benda, '32). Guizzetti ('33) emphasizes the similarity between the lining cells of the cysts and the basophils of the

anterior lobe. Dayton ('26) described transitional cells between the respiratory epithelium of Rathke's pouch and chromophil cells in cyst walls. Rasmussen ('29) pointed out that besides ciliated cells, mucus-producing goblet cells are present in the lining epithelium of such cysts.

Contrary to the above mentioned investigators Stendell ('14), Guizzetti ('25, '27, '28), Kasche ('26) and Pietsch ('30) do not think that all the cysts in this region are derivatives of Rathke's cyst. In fact Pietsch ('30) goes so far as to deny any participation of the original cleft in the formation of these cystic structures, which, according to him, are hollow glandular buds arising from the superior margin of the original hypophyseal cavity. It is possible that both conceptions are partly right in as far as some of the cysts may come from the original cleft while others are derived from hollow glandular buds of the hypophyseal anlage. Cysts which are not lined by a regular epithelium should more likely be considered the result of cellular degeneration (Collin, '22). They probably belong to the anterior lobe and have no direct relationship with the above mentioned cysts.

The cysts of the middle lobe contain a considerable amount of yellow colloid material, the significance of which is not as yet well understood. Rogowitsch (1889) believed it to be a secretion of the granulated cells but Stieda (1890) denied this. There are small colored globules in the blood vessels of this region and in the tissue of the posterior lobe and stalk. These have been considered to be identical with the colloid inside the cysts and the fact that these bodies are found all the way up to the tuber cinereum led to the theory that the hypophysis secretes directly into the nervous system (Collin, '33; Cushing, '33).

Histologically the various colloid accumulations may be largely different. Variations in their optic refractions and dye affinities have been described by Thom ('01, '01 a) and were the subject of many other studies (Kraus, '14; Guizzetti, '25, '27, '28). According to Okamoto ('30) the cystic cavities of the intermediate lobe contain air in children and colloid

does not accumulate until later in life. This conception, however, is most probably based on observations of cysts from which the colloid material fell out in the process of preparation. The cysts may contain a few erythrocytes and desquamated epithelial cells some of which are baso- or eosinophilic. Fat droplets may also be found here either free in the colloid or in phagocytes (Benda, '32; Scheele, '29).

As can be seen from this brief outline of the relevant literature there is no agreement between the various authors concerning the origin and significance of the colloid material. This uncertainty may, at least in part, be ascribed to the fact that the problem has, almost exclusively, been investigated by merely observing normal histological material without any effort to use an experimental approach. In the course of investigations concerning the morphological changes induced by the administration of various hypertonic solutions we observed incidentally that in rats receiving large doses of hypertonic sodium chloride solutions by intravenous infusion, the posterior lobe becomes extremely prominent because the hypophyseal cleft is distended with a large amount of fluid. Under these conditions there appears to be an increased secretion into the pituitary cleft. It was felt that with the aid of such an efficient method to stimulate the secretion of colloid we may be in the position to approach the problem of its secretion experimentally and thus learn more about the mechanism of colloid production.

In our first experiment ten male albino rats weighing 115–140 gm. were used. Under ether anesthesia 6 cc. of a 5% NaCl solution was slowly infused into the jugular vein of each animal. Approximately 10 minutes were necessary for each infusion because if such large amounts of hypertonic solution are more rapidly administered death may ensue during the injection period. All animals were killed 6 hours after the infusion. Mere naked eye inspection sufficed to show that the pituitary was greatly enlarged in all cases. Its borders were rounded and the capsule appeared to be under great tension. The posterior lobe was clearly detached from the anterior by

a greatly distended cyst-like cleft which was filled with clear colorless fluid. The anterior lobe appeared practically normal when viewed macroscopically, but its reddish color suggested pronounced hyperemia.

Histological sections of these pituitaries stained by the method described in a previous publication (Selye and McKeown, '35), revealed that the greatly distended cleft was filled by a rather thin type of colloid which, in the fixed state, appeared to be granular. All three lobes of the pituitary were very hyperemic and this was particularly true of the two so-called "lateral areas" in the anterior lobe (figs. 1 and 2). Degeneration of anterior lobe cells was especially widespread in these regions. However, throughout the tissue of the anterior lobe a large number of basophil cells was in the process of degeneration. In the early stages this change was characterized by a swelling of the cytoplasm which eventually led to a complete degeneration of the cell and transformation of the cytoplasm into a basophilic granular mass similar in appearance and staining reactions to the dilute granular colloid which distended the cleft. It was particularly striking that the nucleus was often still very well maintained at a time when the cytoplasm had already disintegrated. The cells immediately surrounding the small colloid accumulation resulting from the degeneration of a basophil, were then arranged in a manner resembling a lining epithelium. All intermediate stages between such minute cysts and large cystic cavities could be detected throughout the anterior lobes (figs. 3 and 4). Some of these cysts communicated with the main pituitary cleft and appeared to empty their contents into it. Within the colloid of the cleft, desquamated and partly degenerating anterior lobe cells, many of them still retaining the morphological characteristics of basophils, were readily distinguishable. The sinusoids in the anterior and middle lobe tissue immediately bordering upon the cleft were so greatly dilated that their walls protruded deeply into the cleft cavity. In two of the ten animals used in this series the cleft was

filled with blood, a fact which we are tempted to ascribe to the rupture of such engorged vessels.

It appeared of some interest to establish whether isotonic saline solutions would have the same effect. Hence a second experiment was performed on ten male albino rats weighing 120–141 gm. These received injections of as much as 10 cc. of an 0.9% NaCl solution into the jugular vein under circumstances similar to those in the first experiment. At autopsy, 6 hours after the injection, the pituitaries appeared macroscopically normal and histological examination likewise failed to reveal any conspicuous changes such as were seen after treatment with the hypertonic NaCl solution. It must be emphasized, however, that the physiological saline was much more readily excreted through the kidneys than the concentrated solution.

In order to determine whether an inhibition of the renal elimination of intravenously administered hypertonic saline would further intensify the hypophyseal changes caused by the latter, a third experiment was performed on ten male, adult, albino rats weighing 170–178 gm. These were bilaterally nephrectomized and immediately after this operation intravenously injected with 6 cc. of 5% NaCl in the same manner as the animals of the first experiment. They were sacrificed 6 hours after the injection and in these the pituitary changes were even more severe than in the similarly injected intact rats described above. We may therefore conclude that the hypophyseal reaction is not correlated with the actual process of the renal elimination of hypertonic solutions. On the contrary, it is intensified if such elimination is prevented, and hence the osmotic equilibrium of the body becomes more persistently deranged.

In the last experiment we used a vital dye in an attempt to gain further evidence concerning the mechanism of colloid secretion. Five intact and five nephrectomized, male, albino rats weighing 117–136 gm. were intravenously injected with 6 cc. each of an aqueous solution containing 5% NaCl and 0.5% trypan blue. Six hours after the injection all animals

were killed and it was found that while most of the tissues, especially in the nephrectomized rats, were slightly tinged by the blue dye, the cleft of the pituitary contained a particularly large amount of it so that the anterior lobe on the one side and the middle and posterior lobes on the other side were separated by a broad blue dark line (fig. 5). This experiment gives further support to the view that the large amount of fluid seen in the cleft at autopsy had actually been secreted during the few hours after the injection had been given.

From these observations it would appear most likely that the colloid in the anterior lobe cysts, as well as that seen in the cleft itself, is produced by the basophils of the anterior lobe. It arises, at least in part, by a process of holocrine secretion through the disintegration of the basophil cells themselves. In connection with this interpretation it should be mentioned that Ellison and Wolfe ('34) pointed out that in the pituitaries of spayed rats a good deal of colloid tends to accumulate in the cleft, and that this material is similar in its staining reactions to the granules found in the castration cells themselves. With regard to the physiological interpretation of our experiments little can be said until further work demonstrates whether hypertonic solutions of other compounds such as glucose, urea, etc., exert a similar effect. Such experiments are now under way but in the meantime our findings can only be regarded as a lead indicating the existence of certain interrelations between the basophils of the pituitary and the maintenance of the normal NaCl equilibrium or osmotic pressure in the tissues of the body.

#### SUMMARY

Experiments in the rat indicate that intravenous administration of hypertonic sodium chloride solutions causes swelling and eventually degenerative changes in the basophiles of the anterior lobe. Through confluence of the colloid material formed from the body of such basophils small cystic cavities arise throughout the glandular tissue. These cavities, as well as the hypophyseal cleft between the middle and anterior lobe,

gradually increase in size partly through a holocrine secretion of degenerating basophil cell debris and perhaps partly also through a merocrine secretion of the latter and filtration through the greatly engorged anterior lobe sinusoids. Eventually the colloid accumulation, especially in the cleft itself, takes such proportions that the posterior and middle lobes are separated from the anterior lobe by a wide cystic cavity. In its histochemical properties (staining, granulation etc.) the colloid in the cleft resembles that found in the smaller anterior lobe cysts and in the body of individual degenerating basophils.

The observations are interpreted as an indication of a close correlation between anterior lobe cysts and the pituitary cleft. It is believed, furthermore, that the basophils of the anterior lobe are involved in the formation of colloid in both these locations. Since this process of colloid formation is readily and rapidly stimulated by hypertonic salt infusions and not by equivalent amounts of isotonic solutions, it appears possible that the pituitary changes represent a reaction to derangements in the osmotic equilibrium of the body.

#### ACKNOWLEDGMENTS

The expenses of this investigation were defrayed through a grant received from the Rockefeller Foundation.

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## PLATE

## PLATE 1

### EXPLANATION OF FIGURES

1 Section through the pituitary of a normal control rat. Note slit-like cleft between middle and anterior lobe.

2 Section through the pituitary of a rat 6 hours after the administration of hypertonic NaCl solution. Note marked distention of the pituitary cleft by colloid (degree of distention is even greater during life as dehydration occurs in process of fixation). Small light spots throughout the anterior lobe tissue correspond to partly liquefied basophils. Hyperemia and degeneration are most pronounced in the two "lateral areas" of the anterior lobe.

3 Part of anterior lobe and cleft of another rat treated with hypertonic NaCl. Note that the small cysts in anterior lobe tissue and the cleft contain the same type of slightly granular colloid.

4 Two large degenerating basophils in the anterior lobe of a rat treated with hypertonic NaCl. Note that although cytoplasm has completely disintegrated the nucleus is still well preserved.

5 Naked eye view of normal pituitary (top) and that of a rat which had received hypertonic NaCl with addition of trypan blue (bottom). Note the presence of vital dye in the greatly distended cleft.

